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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No,	960296.97290
First Inventor or Application Identifier	Alan D. Attie
Title	Inhibition of Lipoprotein Secretion
Express Mail Label No.	EK290771133US

APPLICATION ELEMENTS

See MPEP Chapter 600 concerning utility patent application contents.

ADDRESS TO: Commissioner for Patents, Box Patent Application, Washington, D.C. 20231

1 [X] Fee transmittal Form (Submit an original and a duplicate for fee processing)

2 [X] Specification [Total 13] *(preferred arrangement set forth below)*
- Descriptive title of the invention
- Cross References to Related Applications
- Statement Regarding Fed Sponsored R&D
- Reference to Microfiche Appendix
- Background of the Invention
- Brief Summary of the Invention
- Brief Description of the Drawings *(if filed)*
- Detailed Description
- Claim(s)
- Abstract of the Disclosure

3 [X] Drawing(s) (35 USC 113) [Total Sheets 2]

4. Oath or Declaration [Total Pages 3]
- a. [X] Newly unexecuted (original or copy)
- b. [ ] Copy from prior Application (37 CFR 1.63(d)) (for continuation/divisional with Box 17 completed)
  [Note Box 5 below]
  - i. [ ] DELETION OF INVENTOR(S) Signed Statement attached deleting inventor(s) named in prior application, see 37 CFR 1.63(d)(2) and 1.33(b).

5 [ ] Incorporation By Reference (useable if Box 4b is checked) The entire disclosure of the prior application from which a copy of the oath or declaration is supplied under Box 4b, is considered as being part of the disclosure of the accompanying application and is hereby incorporated by reference herein.

6. [ ] Microfiche Computer Program *(Appendix)*

7. Nucleotide and/or Amino Acid Sequence Submission *(if applicable, all necessary)*
- [ ] Computer readable Copy
- [ ] Paper Copy (identical to computer copy)
- [ ] Statement Verifying identity of above

ACCOMPANYING APPLICATION PARTS

8 [ ] Assignment Papers (cover sheet & documents)

9 [ ] 37 CFR 3.73(b) Statement (where there is an assignee) [ ] Power of Attorney

10 [ ] English Translation Document *(if applicable)*

11 [ ] Information Disclosure Statement (IDS)/PTO-1449 [ ] Copies of IDS Citations

12 [ ] Preliminary Amendment

13 [X] Return receipt postcard (MPEP 503) *(Should be specifically itemized)*

14 [ ] *Small Entity Statement(s) [ ] Statement filed in prior application Status still proper and desired

15 [ ] Certified copy of priority Document(s) *(if foreign priority is claimed)*

16 [ ] Other:

* A new statement is required to pay small entity fees, except where one has been filed in a prior application and is being relied upon

17. If a CONTINUING APPLICATION, *check appropriate box and supply the requisite information:*

[ ] Continuation [ ] Divisional [ ] Continuation-in-part (CIP) of prior application no. ___/_____

Prior application information: Examiner: Group/Art Unit:

18. CORRESPONDENCE ADDRESS

[ ] Customer Number or Bar Code Label (Insert Customer No. or Attach bar code label) or [X] Correspondence address

NAME	Nicholas J. Seay Quarles & Brady LLP				
ADDRESS	P O Box 2113				
CITY	Madison	STATE	WI	ZIP CODE	53701-2113
COUNTRY	US	TELEPHONE	608/251-5000	FAX	608/251-9166

Name (Print/Type)	Nicholas J Seay	Registration No. (Attorney/Agent)	27,386
Signature		Date	July 21, 2000

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Quarles & Brady LLP

Firstar Plaza
Post Office Box 2113
Madison, Wisconsin 53701-2113
Tel 608.251.5000
Fax 608.251.9166
www.quarles.com

Attorneys at Law in:
Chicago (Quarles & Brady LLC)
Milwaukee
Naples
Phoenix
West Palm Beach

July 21, 2000

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Re: Filing New Patent Application

Dear Sir:

Enclosed for filing please find a new patent application entitled:

INHIBITION OF LIPOPROTEIN SECRETION

by Alan D. Attie
Donald Gillian-Daniel
Paul Bates

The undersigned hereby certifies that this document is being deposited with the United States Postal Service today, July 21, 2000, by the "Express Mail" service, utilizing Express Mail label number EK290771133US, addressed to: Commissioner for Patents, Box Patent Application, Washington, DC 20231.

Please indicate receipt of this application by returning the attached postcard with the official Patent and Trademark Office receipt and serial number stamped thereon.

Respectfully submitted,

Enclosures
QBMAD\223516

INHIBITION OF LIPOPROTEIN SECRETION

CROSS-REFERENCE TO RELATED APPLICATIONS

Not applicable.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH OR DEVELOPMENT

To be determined.

BACKGROUND OF THE INVENTION

Approximately two-thirds of plasma cholesterol in humans is transported on low-density lipoprotein (LDL) molecules. The concentration of LDL in the bloodstream is strongly correlated with the risk of developing premature heart disease to the extent that drugs are designed to lower serum LDL levels. Drugs that reduce the level of LDL in the bloodstream have been shown in numerous clinical trials to be effective in reducing the risk of developing heart disease. The most notable examples are the "statins" (e.g. Zocor, Simvastatin, Lovastatin, Atorvastatin, Pravastatin), drugs that inhibit the activity of 3-hydroxy-3-methyl-glutaryl-coenzymeA reductase, an enzyme in the cholesterol biosynthetic pathway. However, people vary in their responsiveness to these drugs. In particular, some patients with severe forms of hypercholesterolemia are not very responsive to statins or to any other known drug therapy.

An elevation in serum LDL levels can be caused by diminished clearance of LDL particles from the circulation or by increased production of LDL or both. The clearance of LDL from the circulation is largely mediated by the LDL receptor. Thus, patients with familial hypercholesterolemia, a disease caused by LDL receptor mutations, have LDL levels 8-10-fold elevated (in the homozygous form) or 2-4-fold elevated (in the heterozygous form), as compared to patients with normal LDL receptor. This observation provides strong support for the key role of the LDL receptor in LDL

metabolism.

LDL particles are not directly synthesized. Rather, the liver produces very low density lipoprotein (VLDL), which is secreted into the bloodstream. While in the bloodstream, VLDL is converted into LDL. This occurs through the action of lipoprotein lipase (LPL), an enzyme residing on the lumenal surface of the capillary endothelium. LPL catalyzes the hydrolysis of the triglycerides in the VLDL particle, thus shrinking the diameter of the particle and enriching it for cholesterol and cholesterol ester (cholesterol ester is not a substrate for LPL). VLDL also acquires cholesterol ester through the action of cholesterol ester transfer protein (CETP). CETP is in the bloodstream and promotes the transfer of cholesterol ester from HDL to VLDL and the reciprocal transfer of triglyceride from VLDL to HDL. Thus, the actions of LPL and CETP lead to the conversion of a triglyceride-rich particle, VLDL, to a cholesterol-rich particle, LDL.

Excessive secretion of VLDL can lead to high levels of plasma VLDL and/or high levels of plasma LDL. Overproduction of VLDL has been seen as a metabolic consequence of many mutations in the LDL receptor. In addition, a separate metabolic disorder, termed "familial combined hyperlipidemia", also involves the overproduction of VLDL. Consequently, another strategy for dealing with disorders resulting in excessive VLDL (hypertriglyceridemia), excessive LDL (hypercholesterolemia), or both (combined hyperlipidemia) is to interfere with the production and/or secretion of VLDL.

BRIEF SUMMARY OF THE INVENTION

The present invention is, in one aspect, summarized in that a genetic construct includes a promoter operably linked to a protein coding sequence, the protein coding sequence coding for the expression of a fusion protein. The fusion protein includes a low density lipoprotein receptor and a localization domain that acts as a signal for the transport of the protein to the interior of a cell.

The present invention is also summarized in a method which begins with the step of making a genetic construct which includes a protein coding sequence encoding for the expression of a fusion protein. The fusion protein includes a low density lipoprotein receptor and a localization domain which directs localization of the fusion protein to the

interior of a cell in the individual. The construct also includes a promoter effective in the cells of the individual to express the protein coding sequence. The subsequent step is to deliver the genetic construct into the individual.

It is an object of the present invention to provide a methodology to lower serum LDL levels in individuals.

It is another object of the present invention to provide a method to reduce plasma triglycerides in individuals.

Other objects, advantages and features of the present invention will become apparent from the following specification.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

Fig. 1 is a graphical illustration of some of the data from the examples below.

Fig. 2 is a graphical representation of more of the data from the examples below.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based, in part, upon the observation that the functioning of the low density lipoprotein receptor (LDLR) is directly linked to secretion of apolipoprotein B (apoB), the core protein component of VLDL, and hence of LDL. Since LDLR regulates apoB secretion, by facilitating degradation of apoB, it then becomes possible to consider methodologies to use engineered derivatives of native LDLR to inhibit secretion of apoB and thus act to lower serum LDL levels. This same strategy should also result in reduced plasma triglycerides in the treated individuals. It is believed that the LDL receptor and apoB interact early in the secretory pathway, likely in the endoplasmic reticulum (ER) and that degradation of apoB is a result of that interaction. This understanding lead to the design of LDLR constructs intended to be retained inside of the cell, rather than exported to the exterior cell surface, to which native LDLR is normally transported. A derivative of LDLR capable of accomplishing this objective is a truncated form of the LDLR protein to which is appended a sequence capable of localizing the derivative protein in the ER. A truncated form of the LDLR protein, still capable of performing the LDL binding function, but lacking the membrane anchoring region of the native protein, is described in U.S. Patent No. 5,521,071. That

truncated form of the LDLR includes the repeat sequences at the amino terminus of the protein which provide the LDL binding function but does not include the domain associated with membrane binding or the domain associated with O-linked sugars. The truncated LDLR has been shown to be expressed in a conformationally correct form for LDL binding. To that truncated LDLR protein, a localization domain is added. The localization domain is intended not to transport the fusion protein to the cell surface, but to retain the fusion protein inside of the cell. This domain may be as simple as a four amino acid sequence, such as KDEL or HDEL. These tetrapeptides actuate localization of the protein to which they are attached to the ER.

Thus it is envisioned here that gene expression constructs be made which code for the expression of a fusion protein of two parts. One part is a truncated LDLR domain which is truncated so that it is not passed to the extracellular surface but which can effectively bind to LDL. The second part of the fusion protein is a localization domain which is intended to localize the fusion protein inside of the cell producing it. The idea here is that the LDLR is localized inside of the cells, preferably in the ER, where the LDLR will facilitate degradation of apoB. Thus the localization domain that is preferable is one that directs the localization of the fusion protein to an intracellular membrane. Described here are several alternatives for localization domains which will direct the localization of the fusion protein to the ER.

One set of alternatives for the localization domain includes the variants on the Lys-Asp-Glu-Leu (KDEL) amino acid sequence. Proteins tagged with one of these signals are selectively retrieved from a post-ER compartment by a receptor and returned to their normal location. While the KDEL signal is preferred, the following variants are known to be effective in various hosts:

Signal	Species
KDEL, KEEL	Vertebrates, *Drosophila*, *C. elegans*, plants
HDEL	*Saccharomyces. cerevisiae*, *Kluyveromyces lactis*, plants
DDEL, QDEL	*Kluyveromyces lactis*
ADEL	*Schizosaccharomyces pombe* (fission yeast)
SDEL	*Plasmodium falciparum*

Other possible alternatives for intracellular localization signal domains include any cytoplasmic, lumenal (e.g. R402Q variant of human tyrosinase) and transmembrane domains (e.g. CYP2C1) or signals which contain independent ER localization signals such as ribophorin II. Another strategy is to make mutations by amino acid substitution deletion or addition in cytoplasmic, lumenal or transmembrane domains which result in localization in the ER. Some proteins can be retained in the ER if they have impaired or inhibited glycosylation, as from improper folding, such as Kex2p and sialyltransferase II, and that technique could be used to make a localization domain. Sometimes the inhibition of glycosylation can permit interactions with chaperones resulting in cellular retention of proteins as well. Chaperone protein overproduction can also inhibit protein secretion.

The localization domain is produced with the LDLR in such a manner as to maintain the LDLR binding of apoB while permitting the intended localization. The preferred methodology is to locate the localization domain at the carboxyl end of the fusion protein, but amino terminal fusions may work in some instances.

It is envisioned the DNA constructs encoding a fusion protein as described here can be effectively introduced into any mammal to result in lowered levels of apoB. The mechanism described here to facilitate the degradation of apoB is one that operates at a cellular level, and the relevant cellular mechanisms are similar in mammals. For humans, mice are a recognized animal model for testing of LDL lowering strategies.

It is envisioned that a DNA construct encoding the fusion protein of the present invention can be inserted into any expression vector that can cause expression of an inserted protein coding sequence in the host of choice. Such expression construct are transfected into cells which are then altered so as to decrease apoB production. The result is achieved whether or not the cells are treated *in vitro* or *in vivo*, provided only that an expression system appropriate for the host is chosen. As the data below demonstrates, it is possible to introduce an expression construct for the LDLR/localization domain fusion protein into an individual *in vivo* with the result that meaningful decreases in apoB levels are observed. Thus treatment of individuals, as well as cells, is contemplated.

EXAMPLES

In vitro experiments

Vector. The DNA sequence encoding the soluble truncated LDL receptor was obtained from a vector as described in U.S. Patent No. 5,521,071, which encodes a 354 amino acid LRLR. The protein coding sequence was excised from a plasmid pAcLDLR3m, which is described in Dirlam et al., *Protein Expr. Purif.* 8(4):489-500 (1996), using the restriction enzyme Bam HI. The fragment was then inserted into the plasmid pAdBM5 (Quantum Biotechnologies, Inc., Quebec, Canada). The amino acid sequence gln-lys-ala-val-lys-asp-glu-leu-stop (QKAVKDELstop) was introduced into the plasmid beginning at nucleotide position 5257 (amino acid 355) in the $LDLR_{354}$ coding sequence. To do this, two complementary oligonucleotides consisting of the desired sequence were annealed together and ligated into a unique Bgl II site in the plasmid. The sequences encoding the $LDLR_{354}$ and $LDLR_{KDEL}$ were then cloned into the plasmid pAdTRACKCMV, as described in He et al., *Proc. Natl. Acad. Sci. USA* 95(5):2509-14 (1998). This plasmid then contained a cytomegalovirus (CMV) promoter upstream from the $LDLR_{KDEL}$ sequence allowing for efficient protein expression in mouse lymphocytes. This plasmid was used for both the *in vitro* and *in vivo* studies described below.

Preparation of mouse hepatocytes. Hepatocytes were isolated by liver perfusion and seeded at subconfluency in Dulbecco's Modified Eagle Medium (DMEM; GIBCO-BRL supplemented with fetal bovine serum (FBS; 10% v/v; Hyclone), insulin (20mU/ml; Novo Nordisk) and dexamethasone (25nM; Sigma). Cells were left to attach for 4 hours in an incubator at 37°C with 5% CO_2. Following a wash with DMEM, the cells were cultured overnight in DMEM supplemented with 10% FBS and 20 mU/ml insulin. On the following day, cells were transfected with an expression pAdTRACKCMV- $LDLR_{KDEL}$ for a fusion protein of the LDLR truncated receptor with the KDEL localization domain, or $LDLR_{KDEL}$, or with a control plasmid. The transfections were performed using the TransIT-Insecta transfection reagent (Mirus) following the manufacturer's protocol, except that the transfections were performed using 10 mg DNA and 40 ml TranIT-Insecta reagent per 2 ml supplemented with DMEM and 10% FBS in a 60 mm dish of cultured hepatocytes. Transfected

hepatocytes were cultured for an additional 36-48 hour period prior to further experimentation.

Labeling. The hepatocytes were incubated for 1 hour in starve medium before pulse-labeling for 7.5 minutes with radioactive tracer (200 mCi [^{35}S] methionine/cysteine/60 mm dish). The dishes were washed one time with DMEM prior to addition of chase medium (DMEM supplemented with 10mM each of labeled methionine and cysteine and 0.2 mM oleic acid).

Immunoprecipitation. Following the radiolabeling, the media were collected and centrifuged (5 min., 1000 rpm). The resulting media were used for immunoprecipitations. Cells were rinsed three times with ice-cold PBS, scraped into PBS, and collected by centrifugation. The cell pellets were lysed in 200 ml RIPA/1% SDS (150 mM NaCl; 50 mM Tris (pH 7.5); 1% Triton X-100; 0.5% deoxycholate; 1% SDS; 1mM PMSF; 1 mM orthovanadate; 10 mg/ml trypsin inhibitor; 10 mg/ml leupeptin). The mixture was then diluted five times to 1 ml final volume in 150 mM NaCl; 50 mM Tris (pH 7.4); 1 mM PMSF; 1 mM orthovanadate; 10 mg/ml trypsin inhibitor; 10 mg/ml leupeptin. For immunoprecipitations, both the media and the cell lysates were supplemented with 1/5 volume IMB (100 mM Tris (pH 7.4), 25 mM EDTA, 5 mg/ml BSA; 2.5% sodium deoxycholate, 2.5% Triton X-100, 0.01% sodium azide). Antibodies to apoB (polyclonal, rabbit anti-pig LDL) or albumin (polyclonal, rabbit anti-human serum albumin; Sigma) were also added. For the precipitations of albumin, IMB did not contain BSA. After an overnight incubation at 4°C, Protein A-agarose beads (Gibco-BRL) were added and the incubation continued at 4°C overnight. The antibody/ bead slurry was subsequently washed, once with PBB (10 mM phosphate buffer (pH 7.4), 1 mg/ml BSA, 0.01% sodium azide) and once with PB (PBB without BSA). Radiolabeled protein was solubilized in SDS-sample buffer (2% SDS, 20% glycerol, 50mM Tris (pH 6.8), 6 M urea, 1 mM EDTA, 20 mg/ml bromophenol blue), supplemented with 10 mM DTT and 250 mM b-mercaptoethanol, and heated at 65°C for 30 minutes prior to SDS polyacrylamide gel electrophoresis (SDS-PAGE). Specific proteins were visualized by autoradiography and the amounts on unlabeled protein were determined by storage phosphor technology (PhosphorImager, Molecular Dynamics; ImageQuant version 3.3). All data was normalized to cellular protein and total TCA-

precipitable radiation.

Results. The expression of the $LDLR_{KDEL}$ construct in primary hepatocytes resulted in a decrease in the secretion of apoB100-containing lipoproteins. The decrease in apoB100 levels was greater than 50%. Levels of apoB48 were reduced but less than 50%. This reduction was correlated with the expression of the $LDLR_{KDEL}$ protein. The reduction was not observed in the cells transformed with the control plasmid expressing b-galactosidase. Transfection efficiencies ranged between 40 and 60%, suggesting that the reduction of apoB reported here is underestimated. Intracellular levels for triglycerides did not vary between the experimental and control cells.

***In vivo* experiments.**

In a first trial, plasmids encoding either $LDLR_{KDEL}$ or b-galactosidase (control) were injected into a tail vein of mice lacking a functional LDL receptor. Approximately 48 hours after the injection, the mice were fasted for 4 hours and then sacrificed. Plasma from the mice was harvested, diluted 1:1 with PBS, filtered and fractionated using a Pharmacia Sepharose 6 column. The protein profile from that analysis is illustrated in Fig. 1. In Fig. 1, the VLDL/chylomicron remnant, LDL and HDL peaks are identified. Traces are representative for three animals for the control and two for the experimentals. The third experimental animal exhibited no change. Strikingly, the animal with the highest $LDLR_{KDEL}$ expression level, as determined by Western blot analysis, showed an about 50% reduction in plasma cholesterol levels (245.8 mg/dl before injection and 124.6 mg/dl after). Cholesterol levels showed little or no change in plasma from control animals.

The second trial using the $LDLR_{KDEL}$ vector *in vivo* was performed in mice which possessed a wild-type LDL receptor. In this trial the control selected was a plasmid encoding a protein that differs from the KDEL motif by a single amino acid substitution (Ile (140) to Asp). This variant, designated KDEL-ID, was predicted to be deficient in apoB binding and appeared from *in vitro* experimentation to be a suitable control. Mice were injected in a tail vein with 25 mg of DNA coding for either $LDLR_{KDEL}$ or the KDEL-ID variant. Experiments were performed using the Trans-IT In Vivo protocol (Mirus Corporation) according to the manufacturer's instructions. Plasma was harvested approximately 48 hours after injection following a 4 hour fast. The

recovered plasma was diluted 1:1 with PBS, filtered and lipoprotein particles were separated on a Sepharose 6 gel filtration FPLC column (Pharmacia). Cholesterol values for each fraction were determined enzymatically (Sigma). The data is shown in Fig. 2, which represents the mean values for three animals for each treatment, the error bars representing standard error of the mean. The VLDL/chylomicron remnant, LDL and HDL peaks are indicated. This data demonstrates a reproducible lowering of cholesterol levels by about 20%. This result is striking due to the quite low initial VLDL/LDL cholesterol levels in these mice. Additionally, these results may be understated. A mouse HDL particle (HDL-1) co-migrated with LDL and thus may partially mask the effect from the LDL_{KDEL} treatment.

CLAIM OR CLAIMS

I/WE CLAIM:

1. A method for the lowering of serum cholesterol levels in an individual comprising the steps of

making a genetic construct comprising (1) a protein coding sequence encoding for the expression of a fusion protein including a low density lipoprotein receptor and a localization domain which directs localization of the fusion protein to the interior of a cell in the individual, and (2) a promoter effective in the cells of the individual to express the protein coding sequence; and

delivering the genetic construct into the individual.

2. A method as claimed in claim 1 wherein the low density lipoprotein receptor is LDLR354.

3. A method as claimed in claim 1 wherein the localization domain is selected from the group consisting of the amino acid sequences KDEL, KEEL, HDEL, DDEL, QDEL, ADEL and SDEL.

4. A method as claimed in claim 1 wherein the localization domain is KDEL.

5. A method for the lowering of plasma triglyceride levels in an individual comprising the steps of

making a genetic construct comprising (1) a protein coding sequence encoding for the expression of a fusion protein including a low density lipoprotein receptor and a localization domain which directs localization of the fusion protein to the interior of a cell in the individual, and (2) a promoter effective in the cells of the individual to express the protein coding sequence; and

delivering the genetic construct into the cells of the individual.

6. A method as claimed in claim 5 wherein the low density lipoprotein receptor is LDLR354.

7. A method as claimed in claim 5 wherein the localization domain is selected from the group consisting of the amino acid sequences KDEL, KEEL, HDEL, DDEL, QDEL, ADEL and SDEL.

8. A method as claimed in claim 5 wherein the localization domain is KDEL.

9. A DNA construct comprising a promoter operably linked to a protein coding sequence, the protein coding sequence coding for the expression of a fusion protein comprising a low density lipoprotein receptor and a localization domain signaling for the transport of the fusion protein to the interior of a cell.

10. A DNA construct as claimed in claim 9 wherein the low density lipoprotein receptor is LDLR354.

11. A DNA construct as claimed in claim 9 wherein the localization domain is selected from the group consisting of the amino acid sequences KDEL, KEEL, HDEL, DDEL, QDEL, ADEL and SDEL.

12. A DNA construct as claimed in claim 9 wherein the localization domain is KDEL.

13. An artificially constructed fusion protein comprising

a receptor for low density lipoprotein; and

a localization domain signaling for retention of the fusion protein in the interior of a cell.

14. An artificially constructed fusion protein as claimed in claim 13 wherein the low density lipoprotein receptor is LDLR354.

15. An artificially constructed fusion protein as claimed in claim 13 wherein the localization domain is selected from the group consisting of the amino acid sequences KDEL, KEEL, HDEL, DDEL, QDEL, ADEL and SDEL.

16. An artificially constructed fusion protein as claimed in claim 13 wherein the localization domain is KDEL.

ABSTRACT

It has been discovered that the low density lipoprotein receptor (LDLR) degrades the lipoprotein apoB. Based on this observation, an artificial fusion protein has been designed containing an LDL receptor domain attached to a localization domain which causes retention of the fusion protein inside of a cell. The fusion protein is preferably retained in the endoplasmic reticulum of the cell, where the LDLR can degrade apoB. Data shows that the technique is effective in a mammal to reduce serum LDL cholesterol levels.

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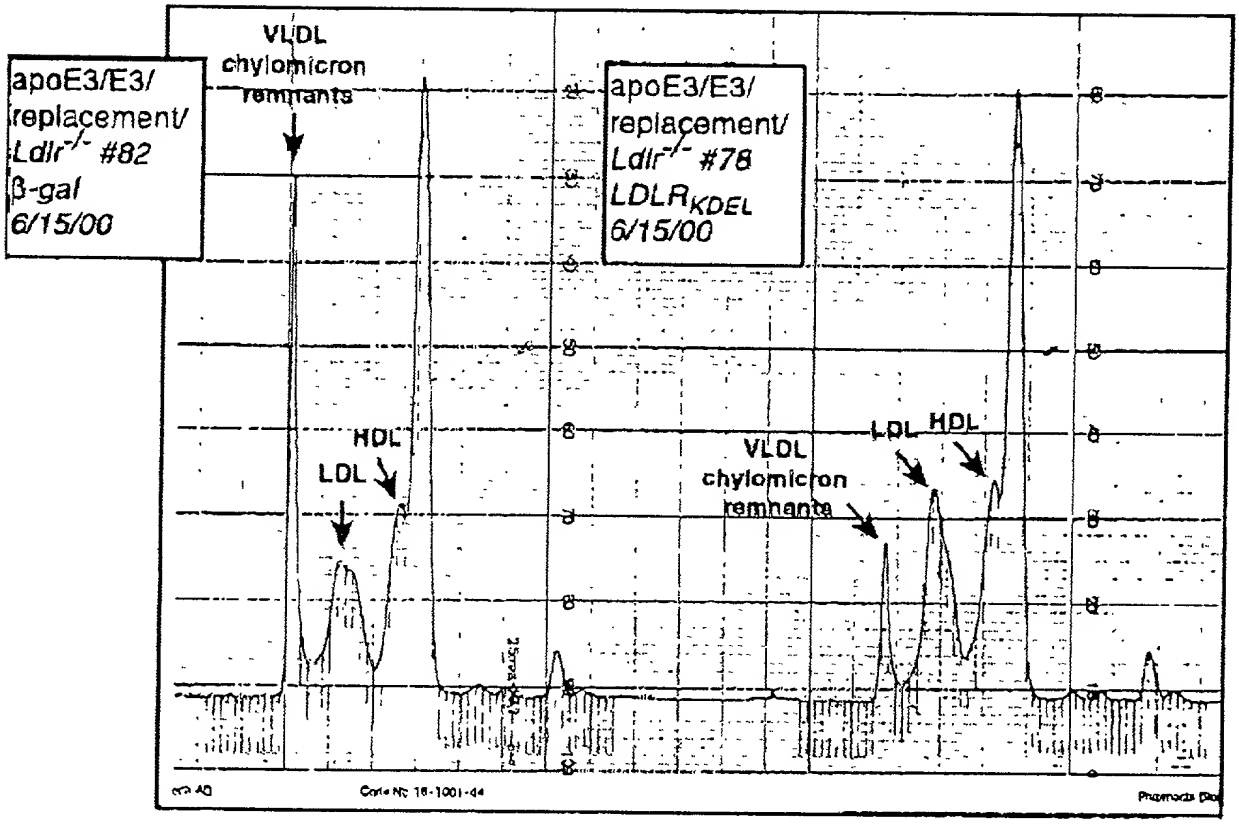
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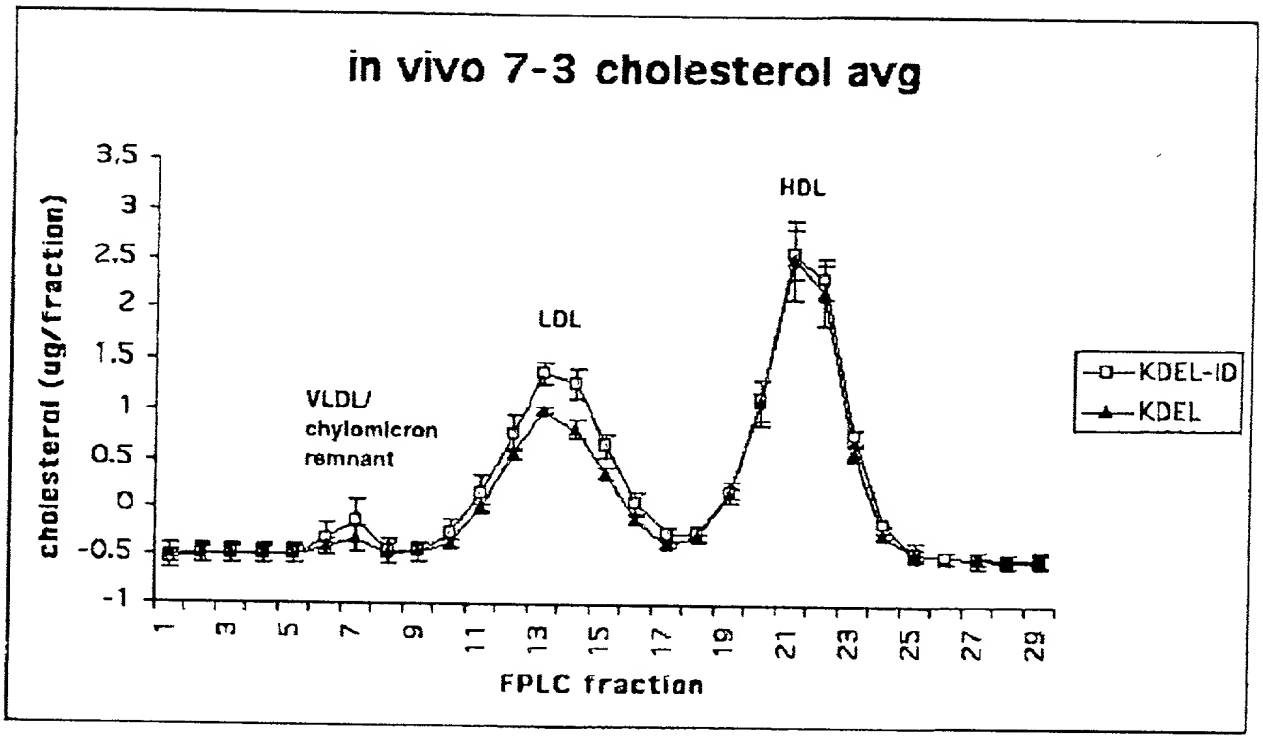
FIG 1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cholesterol (ug/fraction)
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3
2.5
2
1.5
1
0.5
0
-0.5
-1
VLDL/
chylomicron
remnant
LDL
HDL
KDEL-ID
KDEL
1
3
5
7
9
11
13
15
17
19
21
23
25
27
29
FPLC fraction

FIG 2

EXPRESS MAIL LABEL NO. E290771133US

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DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION

[X] Declaration Submitted with Initial Filing OR [ ] Declaration Submitted after Initial Filing

Attorney Docket Number	960296.97290
First Named Inventor	Alan D. Attie
COMPLETE IF KNOWN	
Application Number	
Filing Date	
Group Art Unit	
Examiner Name	

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

INHIBITION OF LIPOPROTEIN SECRETION

(Title of the Invention)

the specification of which

[X] is attached hereto

OR

[ ] was filed on (MM/DD/YYYY) _____ as United States Application Number or PCT International Application Number _____ and was amended on (MM/DD/YYYY) _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56.

I hereby claim foreign priority benefits under Title 35, United States Code §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate or §365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not Claimed	Certified Copy Attached? YES	NO
			[ ]	[ ]	[ ]
			[ ]	[ ]	[ ]
			[ ]	[ ]	[ ]
			[ ]	[ ]	[ ]
			[ ]	[ ]	[ ]
			[ ]	[ ]	[ ]

[ ] Additional foreign applications numbers are listed on a supplemental priority sheet attached hereto:

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)

[ ] Additional provisional application numbers are listed on a supplemental priority sheet attached hereto.

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DECLARATION

I hereby claim benefit under Title 35, United States Code §120 of any United States application(s), or §365(C) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application or PCT international application in the manner provided in the first paragraph of Title 35, United States Code §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

U.S. Parent Application Number	PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number *(if applicable)*

[ ] Additional U.S. or PCT international application numbers are listed on a supplemental priority sheet attached hereto

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and all continuation and divisional applications based thereon, and to transact all business in the Patent and Trademark Office connected therewith:

[ ] Firm Name _____ Customer Number or label _____

OR

[X] List attorney(s) and/or agent(s) name and registration number below

Name	Registration Number	Name	Registration Number
Thomas W. Ehrmann	20,374	David G. Ryser	36,407
Herbert W. Mylius	24,578	Bennett J. Berson	37,094
Barry E. Sammons	25,608	Michael A. Jaskolski	37,551
J. Rodman Steele	25,931	Richard T. Roche	38,599
Nicholas J. Seay	27,386	Mark D. Passler	40,764
George E. Haas	27,642	Stanley A. Kim	42,730
Michael J. McGovern	28,326	Scott D. Paul	42,984
Carl R. Schwartz	29,437	John T. Pienkos	42,997
Gregory A. Nelson	30,577	Daniel G. Radler	43,028
Keith M. Baxter	31,233	Gregory M. Smith	43,136
John D. Franzini	31,356	Steven J. Wietrzny	44,402
Joseph W. Bain	34,290	Steven M. Greenberg	44,725
Robert J. Sacco	35,667	David M. Kettner	45,598
Jean C. Baker	35,433	Adam J. Forman	46,707

[ ] Additional attorney(s) and/or agents named on a supplemental priority sheet attached hereto

Please direct all correspondence to [ ] Customer Number or label _____ OR [X] Fill in correspondence address below

Name	Nicholas J. Seay				
Address	Quarles & Brady LLP				
Address	P O Box 2113				
City	Madison	**State**	WI	**Zip**	53701-2113
Country	USA	**Telephone**	(608)251-5000	**Fax**	(608)251-9166

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Name of Sole or First Inventor: [ ] A petition has been filed for this unsigned inventor

Given	Alan	Middle	D	Family	Attie	Suffix	
Inventor's Signature						Date	

Residence:	Madison	State	WI	Country	US	Citizenship	US
Post Office	1906 Vilas Avenue						
Post Office							
City	Madison	State	WI	Zip	53711	Country	US	Applicant Authority	

[X] [X] Additional inventors are being named on supplemental sheet(s) attached hereto

Please type a plus sign (+) inside this box [+]

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet

Name of Additional Joint Inventor, if any: | A petition has been filed for this unsigned inventor

Given	Donald	Middle		Family	Gillian-Daniel	Suffix	
Inventor's						Date	
Residence:	Madison	State	WI	Country	US	Citizenship	US
Post Office	3497 Milwaukee Street						
Post Office							
City	Madison	State	WI	Zip	53714-2257	Country	US	Applicant Authority	

Name of Additional Joint Inventor, if any: | A petition has been filed for this unsigned inventor

Given	Paul	Middle Initial		Family Name	Bates	Suffix	
Inventor's						Date	
Residence:	Madison	State	WI	Country	US	Citizenship	US
Post Office	6602 Pilgrim Road						
Post Office							
City	Madison	State	WI	Zip	53711	Country	US	Applicant Authority	

Name of Additional Joint Inventor, if any: | A petition has been filed for this unsigned inventor

Given		Middle		Family		Suffix	
Inventor's						Date	
Residence:		State		Country		Citizenship	
Post Office							
Post Office							
City		State		Zip		Country		Applicant Authority	

Name of Additional Joint Inventor, if any: | A petition has been filed for this unsigned inventor

Given		Middle		Family		Suffix	
Inventor's						Date	
Residence		State		Country		Citizenship	
Post Office							
Post Office							
City		State		Zip		Country		Applicant Authority	

Additional inventors are being named on supplemental sheet(s) attached hereto